COMPLEMENT FIXATION IN EXPERIMENTAL TRYPANOSOMIASIS

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It has long been known that the serum of animals infected with trypanosomes will give complement binding with antigens prepared from trypanosomes or the extracts of organs of animals dead from trypanosomiasis. As a part of a general study of experimental trypanosomiasis, we have examined the serum of a number of infected dogs by the complement binding reaction, with the idea of determining: (1) the time of occurrence of the reaction; (2) the relationship of the reaction to the various symptoms and pathologic changes; (3) the specificity of the reaction; (4) its relationship to the Wassermann reaction, and (5) the effect of salvarsan on the reaction.

HISTORICAL

The literature on this subject was abstracted in 1913 by Mohler, Eichorn, and Buck[1] who gave much attention to the preparation of a suitable antigen. They found that extracts of organs of horses dead of dourine did not yield satisfactory antigens but that emulsions of trypanosomes, obtained by laking the blood of rats infected with trypanosomes, yielded a satisfactory antigen. The difficult method of obtaining this antigen (centrifuging, pipetting of the trypanosomes and repeatedly washing) led these observers to prepare a salt solution extract of the spleen of rats just dead of surra. This antigen was generally satisfactory. It was found necessary to prepare this antigen fresh about every 10 days, and to titrate on the day of the test against known positive and known negative sera, in order to guard against the occasional nonspecific fixation and anticomplementary action of such an antigen. Using these precautions the authors uniformly obtained positive results with positive sera, and negative results with negative sera.

Watson,[2] in 1915, reports successful results by the use of an emulsion of trypanosomes as antigen. He likewise states that the extract of the spleen of rats dead of surra yields a satisfactory, but not so sensitive antigen.

PREPARATION OF ANTIGEN

Antigens were prepared by grinding in 30 c.c. of normal salt solution, the spleen of a rat, either heavily infected with T. equiperdum or just dead from such infection. This emulsion was filtered and titrated against two known negative sera, and used in one third the anticomplementary dose, provided the

Received for publication August 18, 1917.

[1] Jour. Agric. Res., 1913-1914, 1, p. 99.
[2] Parasitol., Cambridge, 1915, 8, p. 156.

anticomplementary dose was not over 1:6. If the antigen was anticomplementary, or gave nonspecific fixation with the known negative sera, a phenomea occasionally observed by us and by the previous workers in this field, this antigen was discarded and a fresh one prepared. A fresh antigen was always prepared and titrated on the day of the test. Old antigens were never used. In several of the reactions an antigen composed of an emulsion of T. equiperdum, prepared according to the technic given by Mohler, Eichorn and Buck, was used. This antigen did not appear to be in any way superior to that composed of the spleen extract.

To control the specificity of the reaction, the various sera were all examined against an antigen prepared from the spleen of a normal rat. Wassermann reactions, against crude alcoholic extract of human heart as antigen, were made with all sera.

TABLE 1

COMPLEMENT FIXATION WITH TRYPANOSOME ANTIGEN AND WITH SPLEEN OF NORMAL RAT

Animal	Antigens	April 20	April 21	April 24	April 28
17-64 Splenectomized. Trypanosomes appeared in blood on April 28	Trypanosome spleen	Neg.	Infected	Neg.	++
	Normal spleen	Neg.		Neg.	Neg.
17-65 Splenectomized. Trypanosomes appeared in blood on April 24	Trypanosome spleen	Neg.	Infected	Neg.	++
	Normal spleen	Neg.		Neg.	Neg.
17-66 Trypanosomes appeared in blood on April 24	Trypanosome spleen	Neg.	Infected	Neg.	+++
	Normal spleen	Neg.		Neg.	Neg.
17-67 Trypanosomes appeared in blood on May 2	Trypanosome spleen	Neg	Infected	Neg.	++
	Normal spleen	Neg.		Neg.	Neg.
17-69 Trypanosomes appeared in blood on April 24	Trypanosome spleen	Neg.	Infected	Neg.	+
	Normal spleen	Neg.		Neg.	Neg.
17-72 Trypanosomes appeared in blood on April 24	Trypanosome spleen	Neg.	Infected	Neg.	++
	Normal spleen	Neg.		Neg.	Neg.
17-73 Trypanosomes appeared in blood on April 24	Trypanosome spleen	Neg.	Infected	Neg.	+
	Normal spleen	Neg.		Neg.	Neg.

TECHNIC OF COMPLEMENT FIXATION

The technic used was essentially that described by Snow and Cooper,[3] and outlined in other papers appearing from this laboratory. Any natural anti-sheep hemolysin in the serum to be examined was absorbed by adding 4 c.c. of a 5% suspension of sheep erythrocytes to 1 c.c. of the serum, later removing the cells by centrifuging and using the 20% serum in the test. Three test tubes, containing respectively 0.2, 0.5, and 1.0 c.c. of the dilute serum, together with the usual serum, hemolytic and antigen controls, were run in every instance. A +++ reaction indicates complete or practically complete inhibition of hemolysis with all quantities of serum, a ++ reaction similar inhibition with the 2 larger quantities of serum, and a + reaction such inhibition only in the tube containing 1.0 c.c. of serum.

Sensitized cells were used in all tests. Complement was titrated in the presence of antigen on the morning of the test.

[3] Am. Jour. Med. Sc., 1916, 152, p. 185.

SELECTION OF ANIMALS

The serum of a number of normal dogs were examined against these several antigens—extract of trypanosome spleen, extract of normal spleen, and the Wassermann antigen—in a preliminary reaction. Seven dogs whose sera were not anticomplementary and gave negative reactions with these antigens, were selected for use in this work. Two of these dogs were splenectomized before infection. All dogs were injected with 10 c.c., per kilo of bodyweight, of the blood of a dog heavily infected with trypanosomes.

RESULTS

One very constant finding was noted with respect to the dogs, which from a review of the literature, does not seem to have been observed in the case of

TABLE 1—*Continued*

COMPLEMENT FIXATION WITH TRYPANOSOME ANTIGEN AND WITH SPLEEN OF NORMAL RAT

May 2	May 5	May 9	May 12	May 16	Remarks
+ / Neg.	+ / Neg.	Died May 8			
Died May 2					
+++ / Neg.	++ / Neg.	Serum anticomplementary	Serum anticomplementary	Serum anticomplementary	Treatment with arsenobenzol begun on May 16. See Table 3
+++ / Neg.	++ / Neg.	Serum anticomplementary	Serum anticomplementary	Serum anticomplementary	Died May 22
++ / Neg.	+ / +	Serum becoming anticomplementary	Serum anticomplementary	Splenectomy in May 15	
++ / Neg.	++ / +	Serum becoming anticomplementary	Serum anticomplementary	Serum anticomplementary	Splenectomized May 22
++ / Neg.	+++ / Neg.	++ / Neg.	+++ / Neg.	Serum anticomplementary	Treatment with arsenobenzol begun on May 16. See Table 3

other animals. As the trypanosomes multiplied in the blood of the dogs, and symptoms of trypanosomiasis developed, the sera of the dogs became anticomplementary. This point will be discussed later.

The results of the fixation reactions with trypanosome antigens and antigens of the spleen of a normal rat, are shown in Table 1.

The fixation reaction first appears about 8 days after infection. In all except 1 instance, trypanosomes appeared in the circulating blood before the complement fixation became positive. One dog, on the other hand, gave a positive reaction 4 days before the appearance of trypanosomes in the blood stream, an occurrence in accord with the findings of other observers.

The fixation appeared to be specific. Five dogs gave persistently negative reactions with the extract of a normal rat's spleen, while a positive reaction was obtained with antigens of the spleen of a rat infected with trypanosomes. Two dogs, 17-69 and 17-72, gave a weak reaction with the normal spleen antigen on 1 day, May 2. Three days later both these sera were partially anticomplementary and it seems probable that the weak reaction with the normal spleen antigen may be in some way connected with this phenomena.

RELATIONSHIP WITH THE WASSERMANN REACTION

Owing to the great tendency of dog's sera to give nonspecific fixation with cholesterin antigens, simple alcoholic extract of human heart, of a titer of 1:10, was used in the Wassermann reaction. Table 2 shows the Wassermann reaction of the different sera at different periods after infection. A variable and inconstant fixation of complement with the Wassermann antigen was shown by these sera.

EFFECT OF SALVARSAN ON COMPLEMENT FIXATION

It has been shown by Riquier,[4] and by Schamberg, Kolmer and Raiziss[5] that salvarsan, and its American reproduction arsenobenzol, have a chemotherapeutic effect in trypanosomiasis. After 1 or more injections of arsenobenzol, given intravenously in the dosage of 0.01 gm. per kilo of bodyweight, trypanosomes disappear from the circulating blood of infected animals, there is a prompt clearing up of all symptoms, and the animals return to normal, provided sufficient injections of arsenobenzol are given.

Two dogs have been treated with arsenobenzol in order to determine the effect on the complement fixation reaction. (The arsenobenzol was furnished by Dr. J. A. Kolmer, to whom we express our thanks.) The sera of both these dogs had given strongly positive reactions, and had become anticomplementary prior to the first injection of arsenobenzol. Injections were given on 3 consecutive days, and on every 3rd day thereafter. Sera was collected on the 6th and 9th days after the first injection. These sera, however, were still anticomplementary. One of these dogs died a week later. The remaining dog remained in good condition and the serum was again taken, 21 days after the date of the first injection. This serum was not anticomplementary, and gave completely negative reactions with both the trypanosome and the Wassermann antigens. This result is shown in Table 3. In this one instance, the anticomplementary action and the complement fixation properties with trypanosome and Wassermann antigens were dissipated by treatment with arsenobenzol.

THE ANTICOMPLEMENTARY EFFECT OF TRYPANOSOMES

The anticomplementary phenomena shown by the serum of these dogs at the height of the infection with trypanosomes has already been commented on. This phenomenon disappears following the sterilization of the blood with arsenobenzol. It has seemed possible to us that this complementary action of the serum might be due either to the presence of the large number of trypanosomes in the blood, or to metabolic products freed by the trypanosomes in the blood.

In the hope of casting some light on this problem, we have prepared an emulsion of washed trypanosomes after the method given by the previous workers already mentioned. This emulsion was added in the proportion of 1:10 and 1:20 to the fresh serum of a normal dog. These dilutions were incubated at 38 C. for varying periods, the trypanosomes removed by centrifuging and the serum then tested for anticomplementary action. Table 4 illustrates the results of this experiment.

[4] Ztschr. f. Immunitätsf. u. exper. Therap., 1913, O., 21, p. 92.
[5] Jour. Am. Med. Assn., 1915, 65, p. 2142.

TABLE 2

WASSERMANN REACTIONS

Animal	April 24	April 28	May 2	May 5	May 9	May 12	May 16	June 5
17-64	Neg.	Neg.	Neg.	Neg.	Dead			
17-65	Neg.	+	Dead					
17-66	Neg.	++	++	+	Serum anticomplementary			Neg. (See Table 3)
17-67	Neg.	++	+++	++	Serum anticomplementary			Dead
17-69	Neg.	Neg.	Neg.	+	Serum anticomplementary			Dead
17-72	Neg.	Neg.	+	+	Serum anticomplementary			Dead
17-73	Neg.	Neg.	Neg.	+	Neg.	Serum anticomplementary		Dead

TABLE 3

EFFECT OF SALVARSAN ON COMPLEMENT FIXATION

Animal	Antigen	May 5	May 12	May 16		May 21	May 24	June 5
17-66	Trypanosome spleen	++	Serum anticomplementary		0.01 gms. arsenobenzol on May 16, 17, 18, 21, and 24	Serum anticomplementary		Neg.
	Wassermann	+	Serum anticomplementary			Serum anticomplementary		Neg.
17-73	Trypanosome spleen	+++	+++	Serum anticomplementary		Serum anticomplementary		Died on June 2
	Wassermann	+	Neg.	Serum anticomplementary		Serum anticomplementary		

TABLE 4

ANTICOMPLEMENTARY ACTION OF TRYPANOSOMES

Dilution of Trypanosomes with Fresh Serum								With Salt Solution	
1:10, 2 Hrs. at 38 C., 14 Hrs. in Ice-box	1:10, 4 Hrs. at 38 C., 12 Hrs. in Ice-box	1:10, 16 Hrs. at 38 C.	1:20, 2 Hrs. at 38 C., 14 Hrs. in Ice-box	1:20, 4 Hrs. at 38 C., 12 Hrs. in Ice-box	1:20, 16 Hrs. at 38 C.	Normal Serum 16 Hrs. at 38 C.	Normal Serum 16 Hrs. in Ice-box	1:10, 16 Hrs. at 38 C.	1:20, 16 Hrs. at 38 C.
∓	∓	+	−	−	+	∓	−	+	+

− indicates complete hemolysis, ∓ moderate inhibition of hemolysis, and + complete inhibition of hemolysis.

Trypanosomes in the concentration of 1:10 in fresh serum incubated at 38 C. for 2 and 4 hours, produced a moderate anticomplementary action. In the concentration of 1:20, incubated at 2 and 4 hours, no anticomplementary action was produced. In both the concentration of 1:10 and 1:20 incubated for 16 hours, the trypanosomes rendered the serum completely anticomplementary. A similar effect was rendered in normal salt solution. Straight serum incubated at 38 C. for 16 hours was only slightly anticomplementary, while the same serum kept in the ice-box showed no anticomplementary action.

It seems probable, therefore, that the anticomplementary action developed by the serums of dogs as a result of infection with the trypanosome equiperdum is due to metabolic products liberated by the trypanosomes into the blood.

SUMMARY

Dogs infected with T. equiperdum develop complement fixation with a specific antigen within 8 days after inoculation. An easily prepared and a very satisfactory antigen is the salt solution extract of the spleen of a rat heavily infected with trypanosomes or dead from the infection. The complement fixation usually follows the appearance of trypanosomes in the blood, although it may occasionally precede the appearance of trypanosomes. The complement fixation, however, always antedates the appearance of symptoms.

Dogs infected with trypanosomes frequently give a positive Wassermann reaction.

Within 3 weeks after the appearance of trypanosomes in the blood, the serum of the infected dog becomes strongly anticomplementary. This anticomplementary phenomenon appears to be due to the liberation of anticomplementary substances into the blood by the invading trypanosomes.

The blood is rendered sterile, and all clinical symptoms clear up following the intravenous injection of arsenobenzol, and in the only complete experiment at hand, the anticomplementary action and complement fixation properties with the trypanosome and Wassermann antigens likewise disappeared.